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Reproductive Endocrinology Diseases: Hormone Replacement and Therapy for Peri/Menopause

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1. Introduction

There are many female causes for infertility but the incidence of infertility increases with advancing age. Colombat de L'Isere in a chapter on 'Change of Life' in his "Treatise on the diseases and special hygiene of females" (1845) stated: Compelled to yield to the power of time, women now cease to exist for the species, and hence forward live only for themselves" (Colombat de L'Isere M., 1945). Fortunately, this pessimistic outlook on life after menopause has become outdated. Aiming to standardize terminology The World Health Organization (WHO) assembled in 1996 and the Council of Affiliated Menopause Societies (CAMS) in 1999 (WHO Scientific Group: Research on Menopause in the 1990s, Utian WH, 1999). Commonly accepted terms, including pre-menopause, peri-menopause, menopausal transition, and climacteric, were thought to be too vague to be useful. In July of 2001, the Stages of Reproductive Aging Workshop (STRAW) was held to address the absence of a relevant staging system for female reproductive aging, and to discuss the confusing current nomenclature for the pre-menopause (Soules MR, et al., 2001).

The average age of menopause is 51 and less than 1% of women experience it before the age of 40. Some women undergo premature menopause at a very early age affecting their ability to have children. As more and more women delay child bearing, this life altering condition has become more prevalent. Population aging must be added to population growth as very important social problems. Women in our society get married and have children later in life. Therefore, evaluation of ovarian reserve is critical to understanding a patient's reproductive potential.

Ovulatory disorders are a common cause of infertility. Ovulation is controlled by complex interactions between numerous endocrine hormones including FSH, LH, estradiol, progesterone and others. Menopause is the cessation of the primary functions of the human ovaries, with associated changes in pituitary gonadotropin secretion occurring secondary to

the decline in ovarian sex steroid and protein production. However, increasing evidence suggests that aging is associated with dynamic changes in the hypothalamic and pituitary components of the reproductive axis that are independent of changes in gonadal hormone secretion (Hall JE, et al., 2000). Imbalances in these hormones, or alterations in the "feedback mechanism", can prevent ovulation, or cause it to be irregular.

2. Epidemiology of menopause

Most estimates of age at natural menopause are based on samples of Caucasian women in Western societies. In one large, comprehensive, prospective cohort study of mid-aged, Caucasian U.S. women (the Massachusetts Women's Health Study [MWHS]) the age at natural menopause occurred at 51.3 years, (Gold EB, et al., 2001) confirming prior reports. The Study of Women's Health Across the Nation (SWAN), a multicenter, multiethnic, community-based cohort study of women and the menopausal transition, reported the overall median age at natural menopause to be 51.4 years, after adjustment for other factors (Gold EB, et al., 2001). Studies performed outside the United States suggest that Africans, African Americans, (Bromberger JT, et al., 1997) and Hispanics of Mexican descent experience menopause at an earlier age than Caucasian women, as opposed to Japanese (Tamada T & Iwasake H., 1995) and Malaysian (Ismael NN., 1994) women, who report a similar median age of menopause to women of European descent.

Lower educational attainment and unemployment have been independently associated with earlier age at menopause (Gold EB, et al., 2001; Cramer DW, 1994 et al) and may be markers for elevated bio-psychosocial stress. Women who are separated, divorced, or widowed have been shown to have an earlier menopause than women who are married (Gold EB, et al., 2001). Age at natural menopause for parous women has been reported to occur significantly later than for nulliparous women. (Gold EB, et al., 2001; Anasti JN., 1998; Tibilette MG, et al., 1999; Weel AE, , et al., 1999). Gold et al. and Cramer et al. observed a trend of increasing age at menopause with increasing number of life births, and that prior use of oral contraceptives was associated with earlier age at natural menopause however, a slight prolongation of the reproductive life-span has been associated with oral contraceptive use (Cramer DW, et al., 1994).

The proposed mechanism by which parity and use of oral contraceptives may result in later age at natural menopause involves reducing ovulatory cycles earlier in life and thus preserving oocytes longer, resulting in later menopause (Gold EB, et al., 2001). Some studies show that women with a lower body mass index (BMI) experience an earlier menopause; other studies have not confirmed this finding (Zapantis G & Santoro N., 2002). Environmental toxicants may play a role in early menopause. A large body of literature shows that current smokers tend to experience menopause at an earlier age (1 to 2 years) than non-smokers (Reynaud K,, et al, 2001, Hreinsson JG, et al., 2002, Loffler KA, et al., 2003, Burger HG, et al., 2002) and may have a shorter menopausal transition (Gold EB, 2001, et al).

It has been shown that polycyclic hydrocarbons in cigarette smoke are toxic to ovarian follicles and may lead to their loss and thus an earlier menopause in smokers. Harlow et al. observed that women with a history of medically treated depression had a 20% increased rate of entering peri-menopause sooner than women with no depression history, after adjustment for age, parity, age at menarche, education, cigarette smoking, and BMI. Epidemiology gives answers about populations, whereas clinical medicine deals with

patient samples and individuals. For example, in population-based studies, (Gold EB, et al., 2001) no global increased prevalence of depression has been associated with the menopause transition, whereas in clinical samples, depression around menopause has reportedly increased. Furthermore, symptoms vary among women, and the distinction between populations versus individuals must be made when one is evaluating epidemiologic factors related to menopause.

3. Menopause pathogenesis

The basis of reproductive senescence in women is oocyte/follicle depletion in the ovary. Developmentally, a woman attains her peak oocyte complement at 20 weeks' gestational age. Between 20 and 40 weeks' gestation, two thirds of a woman's oocyte complement is lost, and total oocyte counts drop from a mean of about 6 to 8 million to 1 to 2 million (Zapantis G & Santoro N., 2002).

The most massive wave of atresia (rate of follicle loss) that a woman ever experiences happens before she is born. At the onset of puberty, germ cell mass has been reduced to 300,000 to 500,000 units. Subsequent reproductive aging consists of a steady loss of oocytes through atresia or ovulation and does not necessarily occur at a constant rate. Atresia is an apoptotic process. During the 35 to 40 years of reproductive life, 400 to 500 oocytes will be selected for ovulation. By menopause, only a few hundred follicles remain (Speroff L, et al., 1999).

The relatively wide age range (42 to 58 years) for menopause in normal women seems to indicate that women may be endowed with a highly variable number of oocytes, or that the rate of oocyte loss varies greatly (Soules MR, et al., 2001). Concurrent with the loss of ovarian follicles as a woman transitions to menopause are hormonal changes in the hypothalamic-pituitary-ovarian axis. Follicle-stimulating hormone (FSH) is an established indirect marker of follicular activity; as follicle numbers decline, FSH levels increase (Burger HG, et al., 2002). An elevated level is often the first clinically measurable sign of reproductive aging. Large cross-sectional studies have reported a progressive, quantitative rise in FSH with age (Ahmed-Ebbiary NA, et al., 1994). In the late reproductive years, initial elevations in FSH are most prominent in the early follicular phase of the menstrual cycle but are intermittent and do not occur in every cycle (Klein NA, Soules MR., 1998). This increase is first detectable some years before any clinical indications of approaching menopause are evident (Burger HG, et al., 2002).

The rise in FSH appears to be the result of a decline in inhibin -B, a dimeric protein that reflects the fall in ovarian follicle numbers. Is a small pleomorphic peptide made within the ovarian granulosa and luteinized granulosa cells, which, although assay specificity and sensitivity in ovarian physiology (Burger HG 1993). Inhibin may be an intraovarian regulator although other peptides such as activin and follistatin are more likely paracrine factors (Findlay JK, et al., 1990) but one of its more important functions appears to be feedback suppression of FSH production (Seifer DB, et al., 1996). In reproductive life, inhibin serves to selectively inhibit FSH by binding to receptors on the anterior pituitary (Robertson DM & Burger HG., 2002). Estradiol is stable or even elevated during the earlier menopause transition; closer to the final menstrual period, a decline is clearly observed (Longscope C, et al., 1986).

Findings from the Melbourne Women's Midlife Health Project, a cohort of women followed through the menopause transition, confirm that a decline in inhibin B precedes the increase in FSH and the decline in estradiol that occur later in the transition (Burger HG, et al., 2007).

The remaining follicles are less likely to function normally, which may lead to erratic follicular development and dysregulation of folliculogenesis (Whiteman MK, et al., 2003, Schwingl PJ, et al., 1994). Although FSH and estradiol vary near menopause, steroidogenic enzymes appear to be completely absent in the postmenopausal ovary after all functional follicles are lost (Couzinet B, et al., 2001). Between the ages of 20 and 40 years, concentrations of total testosterone have been reported to fall by about 50% (Zumoff B, et al., 1995). This age-related decline does not change further during the transition years (Burger HG, et al., 2000). Similarly, dehydroepiandosterone (DHEA) and its sulphate, DHEAS, decline with age (Rossmanith W et al., 1991., Santoro N, et al., 1998). Because circulating sex hormone–binding globulin (SHBG) decreases across the menopausal transition, free androgen levels actually rise, as indicated by a small increase in free androgen index (T ÷ SHBG × 100) (Burger HG, et al., 2000). Androstenedione, which remains relatively stable during the transition, is converted to estrone in extra-glandular tissue. This accounts for almost all the estrogen in circulation after menopause.

When ovulation stops, concurrent with a woman's FMP, serum progesterone levels are invariably low (Rannevik G, et al., 1995). Luteinizing hormone (LH) eventually increases, although at a slower rate than FSH. Despite the epidemiologic trend toward elevated FSH and decreased estradiol with progression through the transition, measurement of FSH, inhibin, and estradiol provides at best an unreliable guide to the menopausal status of an individual woman (El-Hage G, et al., 2007; Braunstein GD, et al., 2005). A more rational approach to diagnosing menopause would include an assessment of the longitudinal symptoms of a woman who presents with peri-menopausal complaints (Lobo RA., 1999). Hormone profiles correlate well with symptoms and cycle features (Burger HD, et al., 1995). Thus, if a woman is >45 years old and has had a recent disruption in her menstrual pattern and symptoms suggestive of transient hypo-estrogenemia, it is likely that she has entered her menopausal transition (Santoro N., 2002). That being said, the clinician should take care to rule out other pathologies that can be masked by common complaints associated with the menopausal transition. At minimum, a screening TSH level should be performed, as menstrual irregularity may be the only manifestation of thyroid dysfunction.

4. Regulation of gonadotropins and control of ovarian steroid production

Pituitary gonadotropes synthesize and secrete both LH and FSH. They account for 7% to 15% of anterior pituitary cells. Gonadotropin subunit gene expression is regulated by the frequency of GnRH signal input to pituitary gonadotropes (Haisenleder DJ, et al., 1991). During the menstrual cycle, LH pulse frequency is approximately every 90 minutes in the early follicular phase, 60 to 70 minutes during the late follicular phase, 100 minutes during the early luteal phase, and 200 minutes during the late luteal phase. This variation reflects changes in GnRH pulse frequency, which regulates relative FSH and LH secretion; this, in turn, determines follicle recruitment, development, and ovulation. More rapid GnRH pulse frequencies promote LH secretion, and slower frequencies promote FSH secretion. Although good evidence indicates that changes in GnRH pulse frequency determines differential LH and FSH secretion (Marshall JC, et al., 1993) it is apparent that ovarian steroids and peptide hormones have a major role. Women with hypothalamic stalk section regain their characteristic cycle length when administered an unchanging frequency of exogenous GnRH pulses every 90 minutes.

This indicates that intrinsic rates of follicle development and regression of the corpus luteum, along with their phasing of steroid and peptide hormone production, are major determinants of LH and FSH responses to GnRH. Gonadotropins control the growth and differentiation of the steroid hormone–secreting cells of the ovary, intrinsically linking form and function. A defined sequence of gonadotropin action propels the growth of follicles and the production of steroid hormones. Positive feedback on the pituitary by high concentrations of estrogens leads to the ovulatory surge of LH, which in turn triggers a dramatic differentiation event, resulting in structural reorganization of the pre-ovulatory follicle, release of the ovum, and striking changes in the steroidogenic capacity of the luteinizing cells. Follicular growth, which culminates in ovulation and corpus luteum formation, requires both FSH and LH. Steroidogenic competence of the ovarian follicle is not achieved in the absence of FSH, even if LH is present in abundance. FSH promotes proliferation of the granulosa cells and induces the expression of genes involved in estradiol biosynthesis (Haisenleder DJ, et al., 1991; Kaiser UB, et al., 1997). During the last phase of follicular maturation, when granulosa cells acquire LH receptors, LH is then able to sustain follicular estradiol synthesis. This LH substitution is thought to compensate for the diminished levels of FSH of the late follicular phase consequent to negative-feedback action of estradiol and inhibin. LH action on the granulosa thus rescues the dominant LH-expressing follicle from the fate of atresia. LH stimulation is indispensable for normal ovarian hormone production not only before but also after ovulation. Suppression of LH release leads to a prompt decline in progesterone levels that precede changes in the abundance of mRNAs encoding steroidogenic enzymes or structural changes in the corpus luteum (King JA & Millar RP., 1982).

This acute regulation of ovarian progesterone secretion is controlled by LH via the expression of steroidogenic acute regulatory protein (StAR) messenger RNA (mRNA) and protein, present in both theca-lutein and granulosa-lutein cells throughout the luteal phase are highly expressed in early and midluteal phase, whereas declining StAR mRNA and protein levels are characteristic of late luteal phase. Moreover, StAR protein levels in the corpus luteum are highly correlated with plasma progesterone levels; suppression of LH levels during the midluteal phase markedly decreases plasma progesterone levels and abundance of StAR mRNA transcripts in the corpus luteum (Millar RP, et al., 2004).

4.1 Intra-ovarian control mechanisms

The growth of follicles and the function of the corpus luteum, while under the primary direction of the pituitary, are highly influenced by intra-ovarian factors that modulate the action of gonadotropins. These intra-ovarian factors most likely account for gonadotropin-independent follicular growth, observed differences in the rate and extent of development of ovarian follicles, arrest and initiation of meiosis, dominant follicle selection, and luteolysis. The list of potential paracrine factors that can influence steroid production by theca and granulosa cells is long and diverse. The previous theca cells lacked the aromatase enzyme that is necessary to produce estrogen (Schwanzel-Fukuda M, & Pfaff DW, 1984) so the production of estrogen in granulosa cells indicates presence of aromatase. It includes various growth factors, cytokines, peptide hormones, and steroids such as epidermal growth factor, transforming growth factor β (TGF-β), platelet-derived growth factor, fibroblast growth factors, transforming growth factor α (TGF-α), activins, inhibins, Anti-Müllerian hormone, insulin-like growth factors, estradiol, progesterone, and GnRH (Millar R: 2005; King JA, et al., 2002)

5. The peri-menopausal transition

There is only one marker, menstrual irregularity that can be used to objectively define and establish what is called the peri-menopausal transition. This irregularity will be perceived by patients as skipped menstrual periods or longer durations (about 40 to 60 days) between periods (Harlow SD, et al., 2008) There is no universal pattern; each woman will perceive a change that is her own individual characteristic alteration. Literally means "about or around the menopause." Generally speaking, the term "menopausal transition" is preferred over peri-menopause and climacteric.

The menopause is that point in time when permanent cessation of menstruation occurs following the loss of ovarian activity. Menopause is derived from the Greek words men (month) and pausis (cessation). The years prior to menopause that encompass the change from normal ovulatory cycles to cessation of menses are known as the peri-menopausal transitional years, marked by irregularity of menstrual cycles. Climacteric, an older, more general, and less precise term, indicates the period of time when a woman passes from the reproductive stage of life through the peri-menopausal transition and the menopause to the postmenopausal years (Treloar AE, et al., 1967). Menarche is followed by approximately 5–7 years of relatively long cycles at first, and then there is increasing regularity as cycles shorten to reach the usual reproductive age pattern. In the 40s, cycles begin to lengthen again. The highest incidence of anovulatory cycles is under age 20 and over age 40 (Collett ME, et al., 1954). At age 25, over 40% of cycles are between 25 and 28 days in length; from 25 to 35, over 60% are between 25 and 28 days. The perfect 28-day cycle is indeed the most common mode, but it totalled only 12.4% of Vollman's study cycles. Overall, approximately 15% of reproductive-age cycles are 28 days in length. Only 0.5% of women experience a cycle less than 21 days long, and only 0.9% a cycle greater than 35 days (Munster K, et al., 1992).

Most women have cycles that last from 24 to 35 days, but at least 20% of women experience irregular cycles (Belsey EM & Pinol APY, 1997). When women are in their 40s, anovulation becomes more prevalent, and prior to anovulation, menstrual cycle length increases, beginning 2 to 8 years before menopause (Treloar AE, et al., 1967). Cycles greater than 40 days in length are prevalent in the year before menopause (Ferrell RJ, et al) The duration of the follicular phase is the major determinant of cycle length (Sherman BM, et al) This menstrual cycle change prior to menopause is marked by elevated follicle-stimulating hormone (FSH) levels and decreased levels of inhibin, but normal levels of luteinizing hormone (LH) and slightly elevated levels of estradiol (Buckler HM, et al., 1991; MacNaughton J, et al., 1992; Hee J, et al., 1993; Burger HG, et al., 2000,2008). In the average woman, continuing follicular depletion and declining fertility begin at age 37–38, and menopause follows approximately 13 years later (average age 51). However, in epidemiologic studies approximately 10% of women in the general population become menopausal by the age of 45, probably because they were born with a smaller than normal ovarian follicular pool that is functionally depleted at an earlier age. Menopause occurs when the number of remaining follicles falls below a critical threshold, about 1,000, regardless of age (Treloar AE, 1981).

Recent longitudinal studies of women as they pass through the peri-menopausal transition reveal that estrogen levels do not begin a major decline until about a year before menopause. (Burger HG, et al., 2008; Lasley BL, et al., 2002). Indeed, women experiencing the peri-menopausal transition actually have higher overall estrogen levels, a response that

is logically explained by an increased ovarian follicular reaction to the increase in FSH secretion during these years (Santoro N, et al). Variability in estrogen levels is characteristic of the peri-menopausal transition, with greater variability observed in menstrual cycles that display greater irregularity (Meyer PM, et al). As noted, most women experience a 2- to 8-year period of time prior to menopause when anovulation becomes common (Treloar AE, et al., 1996). During this period of time ovarian follicles continue their rate of loss until eventually the supply of follicles is finally depleted (Gougeon A, et al., 1994). The age-related changes in the endocrine characteristics of the menstrual cycle that result from progressive follicular depletion correlate with a measurable decrease in ovarian volume and in the number of antral follicles observed by trans-vaginal ultrasonography during the early follicular phase (Lass A, et al., 1997; Yong PY, et al., 2003; Frattarelli JL, et al., 2000; Dumesic DA, et al., 2001; Bancsi LF, et al., 2002; Kupesic S, et., 2003). The inverse and tight relationship between FSH and inhibin indicates that inhibin is a sensitive marker of ovarian follicular competence and, in turn, that FSH measurement is a clinical assessment of inhibin (MacNaughton J, et al., 1992; Hee J, et al., 1993). The decrease in inhibin secretion by the ovarian follicles begins early (around age 35), but accelerates after 40 years of age. This is reflected in the decrease in fecundity that occurs with aging. The major decrease in estradiol levels began about 2 years before menopause (Sowers MR, , et al., 2008). Declining levels of inhibin-B and Anti-Müllerian Hormone (AMH) reached a low to non-detectable point about 5 years before menopause (Sowers MR, et al., 2008).

Although the inhibin-B and AMH results are in general agreement with other reports, the exactness of the timing is limited by the fact that the blood samples were obtained from only 50 women in the study. Nevertheless, the Michigan study confirms the validity of AMH as a marker for the ovarian reserve of follicles. Unlike inhibin-B, AMH is not a participant in the feedback relationship between the ovary and the pituitary gonadotropins, rather AMH, a product of granulosa cells, reflects the number of follicles present in the ovaries awaiting FSH stimulation (Visser JA, et al). The variability in these measurements from individual to individual, however, precludes the practical use of these tests to predict with accuracy the future rate of menopause.The peri-menopausal years are a time period during which postmenopausal levels of FSH (greater than 20 IU/L) can be seen despite continued menstrual bleeding, while LH levels still remain in the normal range. Occasionally, corpus luteum formation and function occur, and the peri-menopausal woman is not safely beyond the risk of an unplanned and unexpected pregnancy until elevated levels of both FSH (>20 IU/L) and LH (>30 IU/L) can be demonstrated. The median age for the onset of this transition was 47.5 years. Only 10% of women ceased men-struating abruptly with no period of prolonged irregularity. The peri-menopausal transition from reproductive to post-reproductive status was, for most women, approximately 4 years in duration. In the study by Treloar, the average age for entry into the peri-menopausal transition was 45.1, and the age range that included 95% of the women was 39–51 (Treloar AE, 1996). The mean duration of the peri-menopausal transition was 5.0 years, with a range of 2 to 8 years.

5.1 Endocrine activity of the peri and post-menopausal ovary

As reviewed above, mean estradiol levels are normal or high in peri-menopausal women, and FSH levels are often not suppressed despite these high estradiol levels. These aspects of pituitary-ovarian relationships are contrary to expected physiology. It is proposed that, decreasing ovarian production of inhibin plays a role in the high average estrogen levels documented during the peri-menopause. More specifically, the B subtype of inhibin, a small

peptide made in ovarian granulosa cells, which is known to be stimulated by FSH and, in turn, to suppress FSH, may play a role in the altered physiology of the peri-menopause (Klein NA, et al., 1996, 1998). Increasing evidence suggests that ovarian inhibin plays a role in ovarian folliculogenesis (McLachlan RI, et al., 1986; Hughes EG,,et al., 1992), therefore, new information about inhibin levels and their functional relationships in women in there forties and fifties becomes important.

The peri and post-menopausal ovary contains two different populations of cells with steroidogenic capacity: hilar cells and cortico stromal cells that may represent residual thecal elements (De Roux N, et al., 1999). In vitro studies suggest that the post-menopausal ovary has some steroidogenic potential. Incubation of post-menopausal ovarian stromal slices with pregnenolone yielded progesterone, dehydroepiandrosterone, and testosterone. Incubation of strips of ovarian hilar tissue from postmenopausal women revealed a steroidogenic pattern similar to that of the postmenopausal ovarian stroma. However, the overall amount of steroids produced was substantially greater compared with stroma. Measurable in vitro formation of estradiol by postmenopausal cortical stroma and hilar cells has also been reported (Bertherat J 1998: Ulloa-Aguirre A, et al., 1998).

With increasing age, the adrenal contribution of precursors for estrogen production proves inadequate. In this final stage of estrogen availability, levels are insufficient to sustain secondary sex tissues. Estrogens in peri and post-menopausal women appear to arise almost exclusively from extra-glandular aromatization of androstenedione (Arora KK, et al., 1997). Oophorectomy results in no significant reduction in urinary estrogen excretion by post-menopausal women. However, adrenalectomy after oophorectomy virtually eliminates measurable estrogens from the urine. In vitro studies concluded that the postmenopausal ovarian stroma is unable to aromatize androgens (Everest HM, et al., 2001). However, others have suggested that the post-menopausal ovary may synthesize limited amounts of estrogens, because the concentrations of estradiol and estrone are two times higher in ovarian venous blood than in peripheral blood of post-menopausal women (Illing N, et al., 1999).

There is some evidence that ovarian androgen production in post-menopausal women can be gonadotropin dependent. Administration of hCG to postmenopausal women results in a small increase in the circulating levels of testosterone (Sun YM, et al). Daily injection of hCG causes hyperplasia of the ovarian hilar cells and histochemical evidence suggestive of active steroidogenesis (Tensen C, et al., 1997). Administration of hCG, but not ACTH, resulted in increased androgen but not estrogen production by the ovaries (Wang L, et al., 2001). Binding sites for both LH and FSH were identified in the cortical stroma and in hilar cells (Davidson JS, et al., 1994). Addition of hCG to hilar cells results in increased cAMP formation and steroid biosynthesis, indicating preserved responsiveness to gonadotropins. Taken together, these observations suggest that ovarian androgen biosynthesis of the post-reproductive ovary is at least partially gonadotropin dependent.

The post-menopausal ovary is occasionally involved in pathologic endocrine activity. Stromal hyperplasia can occur, with the ovary enlarging with hyperplastic stromal nodules consisting of lipid-rich luteinized cells that resemble theca interna. The ovaries with stromal hyperplasia produce large amounts of androstenedione, resulting in hirsutism and virilisation (Vrecl M, et al., 1998). Hilar cells can give rise to functional hilar cell tumors, which produce excess amounts of androgens, leading to virilisation (Pawson AJ, et al., 1998; Blomenrohr M, et al., 1999; Heding A, et al., 2000). Signs and symptoms of estrogen excess may also be evident in circumstances of significant peripheral aromatization.

6. Treatment of anovulation

If no primary pathology is apparent, or if the primary pathology has been treated appropriately without restoration of normal endocrinology, treatment options lie between estrogen replacement (or the oral contraceptive in a younger woman) to prevent osteoporosis and ovulation induction to restore fertility. Estrogen antagonists usually are ineffective in inducing ovulation in progestogen-negative women, and treatment with pulsatile GnRH or gonadotropin treatment is normally required. (Elizur SE, et al., 2005). Treatment with pulsatile GnRH involves the woman carrying a small portable pump but has the important advantage of a lower multiple pregnancy rates than is seen with gonadotropin treatment. Women with a low LH concentration (<4 IU/L) require treatment with combined gonadotropin treatment regimens that include both FSH and LH bioactivity. The older urinary gonadotropin preparations have sufficient LH bioactivity, but when a recombinant FSH is used, it must be supplemented with recombinant LH or with a low dosage of urinary hCG.

6.1 Ovulation inductions and assisted reproduction

The reproductive period in women is characterized by their ability to ovulate. Ovulation, the release of an oocyte within the peritoneal cavity, follows rupture of a dominant follicle, developed in response to stimulation by endogenous gonadotropins. In the presence of normal fallopian tubes, the released oocyte will be able to interact with spermatozoa ascending the female genital tract. This may lead to production of the zygote and establishment of pregnancy if implantation occurs. When ovarian activity is disrupted, no ovulation takes place and, as a consequence, achievement of pregnancy is not feasible.

Ovulation induction refers to exogenous direct or indirect stimulation of the ovary with the aim of alleviating sub-fertility due to anovulation. Ovulation induction should be differentiated from *reestablishment of ovulation,* which occurs after treatment of conditions interfering with the normal function of the hypothalamic-pituitary-ovarian (HPO) axis. These include weight and eating disorders, thyroid dysfunction, hyper-prolactinemia, and excess exercise. It should also be differentiated from *enhancement of ovulation.* This is usually performed in ovulatory women with unexplained infertility in the hope of increasing the probability of pregnancy. More important, ovulation induction must be differentiated from superovulation for in vitro fertilization (IVF), in which the aim of ovarian stimulation is to induce multi-follicular development. This leads to the retrieval of multiple oocytes and thus allows the selection of the morphologically best embryo(s) for replacement. In IVF, follicular rupture is not necessary, because oocytes are collected by trans-vaginal aspiration (Pelinck MJ, et al., 2001; Castelo-Branco A, et al., 2004; Kolibianakis E, et al., 2002,2003).

6.2 Ovarian stimulation regimens

The ideal ovarian stimulation regimen for IVF should have a low cancellation rate, minimize drug costs, risks and side effects, require limited monitoring for practical convenience, and maximize singleton pregnancy rates. Numerous regimens have been described, ranging from no stimulation (natural cycles), to minimal stimulation (clomiphene citrate) or mild stimulation (sequential treatment with clomiphene citrate and low dose exogenous gonadotropins), to aggressive stimulation (high dose exogenous gonadotropins, alone or in combination with a gonadotropin-releasing hormone agonist or antagonist). Ovarian stimulation has been a basic element of IVF for more than 25 years, but concerns about

multiple pregnancies and the costs of IVF have sparked renewed interest in natural cycle IVF and mild stimulation regimens (Nargund G, et al., 2001)

7. Natural cycle

The first birth resulting from IVF derived from a single oocyte collected in a natural ovulatory cycle (Steptoe PC, & Edwards RG, 1978). Compared to stimulated IVF cycles, natural cycle IVF offers a number of attractive advantages. Natural cycle IVF involves only monitoring the spontaneous cycle and retrieving a single oocyte before the midcycle LH surge occurs. It is physically less demanding, requires little or no medication, decreases costs by 75–80%, (Aboulghar MA, et al., 1995; Nargund G, et al., 2001) and all but eliminates risks for multiple pregnancy and ovarian hyper-stimulation syndrome (OHSS). The chief disadvantages of natural cycle IVF are high cancellation rates due to premature LH surges and ovulation, and the comparatively low success rate, which is approximately 7% (Pelinck MJ, et al., 2002). When oocyte retrieval is based on detection of the mid-cycle rise in LH, careful and frequent monitoring is required and procedures are difficult to schedule efficiently.

Alternatively, exogenous human chorionic gonadotropin (hCG) can be administered when the lead follicle reaches a size consistent with maturity, thereby better defining the optimum time for oocyte retrieval (Nargund G, et al., 2001). Adjuvant treatment with a GnRH antagonist also can be used to prevent a premature LH surge, but requires "add-back" treatment with exogenous FSH, and success rates are still quite low, ranging up to 14% per cycle in non-randomized trials (Castelo-Branco A, et al., 2004; Kolibianakis E, et al., 2002, 2003; Weghofer A, et al., 2004; Elizur SE, et al., 2005). In one large cohort study involving 844 treatment cycles in 350 good prognosis patients, the cancellation rate was 13%, the pregnancy rate was 8% per cycle and the cumulative pregnancy rate after three "modified natural IVF cycles" was 21% (Pelinck MJ, et al., 2002). In a cohort of infertile couples with male factor infertility, success rates in modified natural cycles have reached as high as 13% per cycle, with a cumulative pregnancy rate of 44% after six treatment cycles (Verberg MF, et al., 2006).

8. Clomiphene citrate

Clomiphene citrate (CC) was the first method of ovarian stimulation used in IVF, (Quigley MM, et al., 1984) but now has been almost entirely replaced by more effective stimulation regimens using human menopausal gonadotropins (hMG) or FSH, in combination with a GnRH agonist or antagonist (Macklon NS, et al., 2006). Clomiphene (100 mg daily) usually is administered for 5–8 days, beginning on cycle day 3, and induces development of two or more follicles in most normally ovulating women, (Dickey RP, et al., 1998; Messinis IE & Milingos SD 1998; Ingerslev HJ, et al., 2001) although egg yields 1–3, are only slightly greater than in un-stimulated cycles and substantially lower than in cycles stimulated with exogenous gonadotropins (Ingerslev HJ, et al., 2001; Branigan EF & Estes MA 2000; MacDougall MJ, Tan SL, Hall V, et al., 1996).

Cycle cancellation rates are somewhat lower than in natural cycles and the numbers of oocytes retrieved, embryos transferred, and pregnancy rates are greater. As in natural stimulates multi-follicular development more effectively than treatment with Clomiphene alone (Corfman RS, et al., 1993; Dor J, et al., 1992). Drug costs and monitoring requirements

are moderately cycles, exogenous hCG is administered when the lead follicle reaches mature size and a GnRH antagonist can be used to prevent a premature endogenous LH surge. Sequential treatment with clomiphene (100 mg daily for 5 days) and modest doses of exogenous gonadotropins (150–225 IU daily beginning on the last day of clomiphene treatment or the day after) higher, but still substantially less than in standard stimulation regimens involving higher dose gonadotropin treatment after down-regulation with a long-acting GnRH agonist (described below) (Weigert M, et al., 2002; Dhont M, et al., 1995). In one comparative trial, higher cancellation rates and lower pregnancy rates were observed in sequential clomiphene/gonadotropin cycles (Dhont M, et al., 1995). In another, the sequential stimulation regimen yielded fewer oocytes and embryos, but pregnancy rates were similar and the risks of ovarian hyper-stimulation syndrome (OHSS) were lower (Weigert M, et al., 2002).

In a randomized trial, sequential clomiphene/gonadotropin stimulation and GnRH antagonist treatment yielded a pregnancy rate comparable to that achieved with a more aggressive standard treatment protocol, (Lin YH, et al., 2006) confirming the results of two earlier retrospective studies, (Fiedler K & Ludwig M 2003; Williams SC, et al., 2002), but contrasting with those of another observing lower pregnancy rates (Mansour R, et al., 2003).

9. GnRH agonist "flare" gonadotropins stimulation protocol

The "short" or "flare" protocol is an alternative stimulation regimen designed to exploit both the brief initial agonistic phase of response to a GnRH agonist and the suppression that results from longer-term treatment (Padilla SL, et al., 1996; Garcia JE, et al., 1990). In a typical standard short protocol, leuprolide acetate (1.0 mg daily) is administered on cycle days 2–4, continuing thereafter at a reduced dose (0.5 mg daily), and gonadotropin stimulation (225–450 IU daily) begins on cycle day 3. Later adjustments in the dose of gonadotropin stimulation, if needed, are based on response and indications for hCG administration are the same as in the long protocol (described above). An early meta-analysis including seven clinical trials comparing the short and long GnRH agonist treatment regimens determined that the two protocols yielded similar cancellation and pregnancy rates: (Hughes EG, et al., 1992).

A 2000 systematic review including 22 trials concluded that pregnancy rates achieved with the long protocol were superior to those using the flare regimen (OR=1.27, CI=1.04–1.56) overall, (Daya S., 2000), but the analysis did not control for diagnosis and other prognostic factors and results may not apply to all women, or to poor responders in particular. Whereas some have observed improved follicular response and lower cycle cancellation rates in poor responders treated with a flare protocol, pregnancy and live birth rates remained low (Karande V, et al., 1997; Karacan M, et al., 2001). Decreased scheduling flare exibility is a distinct disadvantage of the flare is protocol, unless the onset of menses is controlled by preliminary treatment with an OC. The regimen also can result in a significant increase in serum progesterone and androgen levels, presumably resulting from late corpus luteum rescue, (San Roman GA, et al., 1992) which may adversely affect oocyte quality and fertilization and pregnancy rates (Loumaye E, et al., 1989). The "OC micro-dose GnRH agonist flare" stimulation regimen is a variation of the standard short protocol involving 14–21 days of preliminary ovarian suppression with an OC (one pill daily), followed by micro-dose leuprolide treatment (40 µg twice daily) beginning 3 days after discontinuation of OC treatment, and high-dose gonadotropin stimulation (300–450 IU daily) starting on day 3 of

leuprolide therapy. Indications for later gonadotropin dose adjustments and hCG administration are the same as in other stimulation regimens. Its primary advantage over the standard short protocol is that it does not induce any increases in serum progesterone or androgen concentrations, possibly because the doses of GnRH agonist administered are much lower, but likely also because preliminary OC treatment all but eliminates the possibility there may be a corpus luteum left to respond (Gonen Y, et al., 1990; Cedrin-Durnerin et al., 1996). The OC-micro-dose GnRH agonist flare protocol may be useful in previous poor responders, in whom it can stimulate increased endogenous FSH release and may yield lower cancellation rates and higher peak serum estradiol levels, transfer rates and pregnancy rates (Surrey ES, et al., 1998; Scott RT et al., 1994) GnRH Antagonist Gonadotropin Stimulation Protocol.

The introduction of GnRH antagonists into clinical practice provided another option for ovarian stimulation in ART. In contrast to the long-acting agonists, which first stimulate and later inhibit pituitary gonadotropin secretion by desensitizing gonadotropes to GnRH via receptor down-regulation, the antagonists block the GnRH receptor in a dose-dependent competitive fashion and have no similar flare effect (Matikainen T, et al., 1992; Reissmann T, et al., 1974,1995) gonadotropin suppression is almost immediate. GnRH antagonists offer several potential advantages over agonists. First, the duration of treatment for an antagonist is substantially shorter than for an agonist. Since its only purpose is to prevent a premature endogenous LH surge and its effects are immediate, antagonist treatment can be postponed until later in follicular development (after 5–6 days of gonadotropin stimulation), after estradiol levels are already elevated, thereby eliminating the estrogen deficiency symptoms that may emerge in women treated with an agonist (Olivennes F, et al., 2002).

Second, because any suppressive effects that agonists may exert on the ovarian response to gonadotropin stimulation also are eliminated, the total dose and duration of gonadotropin stimulation required is decreased (Olivennes F, et al., 2002; Albano C, et al., 2000). For the same reason, GnRH antagonist stimulation protocols may benefit women who are poor responders when treated with a standard long protocol (Olivennes F, et al., 2003; Akman MA, et al., 2001). Third, by eliminating the flare effect of agonists; GnRH antagonists avoid the risk of stimulating development of a follicular cyst. Finally, the risk of severe OHSS associated with use of antagonists also appears lower than with agonists. GnRH antagonists have some potential disadvantages. When administered in small daily doses, strict compliance with the prescribed treatment regimen is essential (Olivennes F, et al., 2002).

Antagonists suppress endogenous gonadotropin secretion more completely than agonists. Whereas the low levels of LH observed during agonist treatment are usually sufficient to support normal follicular steroid-genesis during stimulation with uFSH or rFSH, the even lower concentrations in women treated with an antagonist may not be. Indeed, serum estradiol levels may plateau or fall when antagonist treatment begins (Olivennes F, et al 2002;, de Jong D, et al., 2001). Although follicular growth appears unaffected, most prefer to add or substitute a low dose of hMG (75 IU) at the same time if it was not already part of the stimulation regimen. Evidence also suggests that pregnancy rates in antagonist treatment cycles may be modestly lower than in cycles using agonists in the long protocol (Al-Inany et al., 2006).

The two GnRH antagonists available for clinical use, ganirelix and cetrorelix, are equally potent and effective. For both, the minimum effective dose to prevent a premature LH surge is 0.25 mg daily, administered sub-cutaneously (Albano C, et al., 1997). Either can be administered in a series of small daily doses (0.25 mg). The treatment protocol may be fixed

and begin after 5–6 days of gonadotropin stimulation, (Albano C, et al., 1997; Diedrich K, et al., 1994), or tailored to the response of the individual, starting treatment when the lead follicle reaches approximately 13–14 mm in diameter. The individualized treatment regimen generally requires fewer total doses and may yield better overall results (Ludwig M, et al., 2002). Alternatively, a single larger dose of cetrorelix (3.0 mg) will effectively prevent an LH surge for 96 hours. If given on day 6–7 of stimulation, the interval of effective suppression will encompass the day of hCG administration in most women (75–90%); the remainder may receive additional daily doses (0.25 mg) as needed, ending on the day of hCG treatment (Olivennes F, et al 1995; Olivennes F, et al., 2000; Olivennes F, et al., 2003). The single dose antagonist treatment regimen also can be withheld until the lead follicle reaches 13–14 mm in diameter (Fanchin R, et al., 2003,2005).

A common variation of the antagonist stimulation regimen uses preliminary treatment with an OC to control the onset of menses, typically ending approximately 5 days before the scheduled start, which also may help to synchronize the follicular cohort before stimulation begins. Another variation advocated for poor responders uses micronized estradiol (2 mg twice daily, administered orally, beginning on day 21 of the preceding cycle) to suppress FSH during the late luteal phase for the same purpose, ending on the day before gonadotropins stimulation begins, (Fanchin R, et al 2003, 2005), or continuing through the first 3 days of gonadotropin stimulation (Hill MJ, et al., 2009). The improved follicular dynamics observed are similar to those achieved by down-regulation with a GnRH agonist in the long protocol. The rebound increase in endogenous FSH levels that follows the discontinuation of estradiol treatment also may synergize with exogenous gonadotropins to promote multi-follicular development (de Ziegler D, et al., 1998; Fanchin R, et al., 2003). Results of a number of early trials comparing a fixed antagonist treatment protocol to the standard long protocol suggested that the two stimulation regimens yielded similar pregnancy rates (Albano C, et al., 2000; Olivennes F, et al., 2000); The European Middle East Orgalutran Study Group, 2001; Fluker M, et al., 2001). However, a 2006 systematic review and meta-analysis including 27 trials comparing different antagonist stimulation protocols with the long GnRH agonist protocol observed a significantly lower clinical pregnancy rate (OR=0.84, CI=0.72–0.97) and ongoing pregnancy/live birth rate (OR=0.82, CI=0.69–0.98). Overall, the total dose and duration of gonadotropin stimulation required, peak serum estradiol levels, and the number of follicles and oocytes were lower in antagonist cycles.

The explanation for the modestly lower pregnancy rates observed in antagonist treatment cycles is not clear. It is possible, but unlikely, that GnRH antagonists may have adverse effects on oocytes, embryos, or the endometrium (Hernandez ER 200; Ortmann O, et al., 2001). It is far more likely that early results reflected inexperience and improved with time and further refinements in the treatment regimen like those described above. Many of the advantages originally envisioned for GnRH antagonists already have been realized. Whether antagonists ultimately will replace agonists and become the standard ovarian stimulation regimen in ART cycles remains to be seen, but their place in the therapeutic arsenal already is firmly established. Whereas a single bolus injection of an agonist (leuprolide 0.5 mg, triptorelin 0.2 mg) triggers a physiologic LH surge that lasts less than 24 hours, hCG levels remain elevated for several days and stimulate markedly higher estradiol and progesterone concentrations (Fauser BC, et al., 2002).

The antagonist treatment regimens currently in use have potential disadvantages for women with PCOS. Their tonically elevated LH levels will remain high until antagonist treatment begins. Consequently, LH levels may rise prematurely, particularly if antagonist treatment

is withheld until the lead follicle reaches 14 mm or more. Moreover, evidence indicates that increased LH exposure during early follicular development may be detrimental and predispose to lower pregnancy rates (Kolibianakis E, et al., 2002; Kolibianakis EM, et al., 2003; Kolibianakis EM, et al., 2003; Kolibianakis E, et al., 2003). In theory, pre-treatment with an OC might prove quite useful by suppressing LH and androgen levels before stimulation begins, decreasing exposure during early follicular development and the risk of rising LH levels before antagonist treatment starts. Preliminary OC suppression and later antagonist treatment may help to limit the follicular response to gonadotropin stimulation while preserving the option to use an agonist to trigger final oocyte maturation. These considerations simply serve to illustrate that GnRH antagonists are not a panacea and are not necessarily the best choice even for women with PCOS. Antagonist stimulation protocols are advocated for poor responders, primarily because they avoid the suppressive effects that agonists can have on follicular response and can prevent the premature LH surges observed commonly in women stimulated with gonadotropins alone (Surrey ES & Schoolcraft WB., 2000). However, evidence is insufficient to indicate they yield results consistently better than other stimulation regimens (Pandian Z, et al., 2010; Centers for Disease Control and Prevention, Atlanta, GA, 2009).

10. Ovarian reserve

The concept of ovarian reserve, generally defined as the size and quality of the remaining ovarian follicular pool, and the various methods for its measurement. The total number of oocytes in any given women is genetically determined and inexorably declines throughout life, from approximately 1–2 million at birth, to about 300,000 at puberty, 25,000 at age 40, and fewer than 1,000 at menopause (Battaglia DE, et al., 1996; Faddy MJ & Gosden RG, 1996). The rate of follicular depletion is not constant, but increases gradually as the number of follicles remaining decreases (Nilsson E, et al., 2007; Adhikari D & Liu K, 2009; Da Silva-Buttkus P, et al., 2009; Coxworth JE, & Hawkes K, 2010). As the size of the remaining follicular pool decreases, circulating inhibin-B levels (derived primarily from smaller antral follicles) decrease, resulting in lower levels of feedback inhibition and a progressive increase in serum follicle-stimulating hormone (FSH) levels, most noticeably during the early follicular phase (Klein NA, et al., 1996; Welt CK, McNicholl DJ, Taylor AE, et al; Hale GE, et al., 2007 ; Knauff EA, et al., 2009; Burger HG, et al., 2008). Increasing inter-cycle FSH concentrations stimulate earlier follicular recruitment, resulting in advanced follicular development early in the cycle, an earlier rise in serum estradiol levels, a shorter follicular phase, and decreasing overall cycle length (Klein NA, et al., 2002; de Koning CH, et al., 2008).

The physiology of reproductive aging provides the foundation for all contemporary tests of ovarian reserve. In clinical practice, the basal early follicular phase (cycle day 2–4) FSH level is the most common test, but antimüllerian hormone (AMH) and antral follicle count are alternatives having significant potential advantages. As basal FSH levels increase, peak estradiol levels during stimulation, the number of oocytes retrieved, and the probability for pregnancy or live birth decline steadily (Pearlstone AC, et al., 1992; Scott Jr RT & Hofmann GE, 1995; Bukman A, & Heineman MJ, 2001). With current assays (using IRP 78/549), FSH levels greater than 10 IU/L (10–20 IU/L) have high specificity (80–100%) for predicting poor response to stimulation, but their sensitivity for identifying such women is generally low (10–30%) and decreases with the threshold value (Broekmans FJ, et al., 2006) . Although

most women who are tested have a normal result, including those with a diminished ovarian reserve (DOR), the test is still useful because those with abnormal results are very likely to have DOR. In a 2008 study, an FSH concentration above 18 IU/L had 100% specificity for failure to achieve a live birth (Scott Jr RT, et al., 2008). The basal serum estradiol concentration, by itself, has little value as an ovarian reserve test (Hazout A, et al., 2004; Eldar-Geva T, et al., 2005; McIlveen M, et al., 2007), but can provide additional information that helps in the interpretation of the basal FSH level. An early elevation in serum estradiol reflects advanced follicular development and early selection of a dominant follicle (as classically observed in women with advanced reproductive aging), and will suppress FSH concentrations, thereby possibly masking an otherwise obviously high FSH level indicating DOR. When the basal FSH is normal and the estradiol concentration is elevated (>60–80 pg/mL), the likelihood of poor response to stimulation is increased and the chance for pregnancy is decreased (Evers JL, et al., 1998; Buyalos RP, al., 1997). When both FSH and estradiol are elevated, ovarian response to stimulation is likely to be very poor. Antimüllerian hormone (AMH) derives from pre-antral and small antral follicles. Levels are gonadotropin-independent and vary little within and between cycles (Fanchin R, et al., 2005; Tsepelidis S, et al., 2007; Hehenkamp WJ, et al., 2006). The number of small antral follicles correlates with the size of the residual follicular pool and AMH levels decline progressively with age, becoming undetectable near the menopause (Sowers MR, et al., 2008; van Rooij IA, et al., 2004; van Rooij IA, et al., 2005).

10.1 Oocyte and ovarian tissue cryopreservation

Each year, cancer occurs in approximately 100 per 100,000 women under age 50 in the United States. Chemotherapy and radiation therapy for malignant and non-malignant systemic disease very often results in ovarian failure. Women with cancer and other serious illnesses requiring treatments that pose a serious threat to their future fertility have relatively few options. In some cases, the ovaries may be moved out of the radiation field. Treatment with GnRH agonists has been suggested as a way to protect the gonads from the insult of chemotherapy, but there is no convincing evidence for its efficacy. Although embryo banking is effective, the time required for stimulation and retrieval are often prohibitive. With recent advances in cryobiology, oocyte and ovarian tissue cryopreservation hold promise as methods to preserve reproductive potential (Shaw JM, et al., 2000).

10.2 Oocyte cryopreservation

Although the first pregnancy resulting from oocyte cryopreservation was reported in 1986, (Chen C, 1986), success rates achieved with the technology were historically very low, and only recently improving. The primary obstacle was the poor survival of oocytes, which are fragile due to their size, high water content, and chromosomal arrangement; the meiotic spindle is easily damaged by intracellular ice formation during freezing or thawing (Shaw JM, et al., 2000). Germinal vesicle stage oocytes are hardier, (Boiso I, et al., 2002), but progress with in vitro maturation of immature oocytes has been slow. Another obstacle was hardening of the zona pellucida, which interfered with normal fertilization. The improved survival of cryopreserved oocytes today relates primarily to modifications in the sucrose and sodium concentrations in traditional “slow-freeze” protocols, (Fabbri R, et al., 2001; Stachecki JJ & Willadsen SM, 2000; Bianchi V, et al., 2007; De Santis L, et al., 2007), changes.,

in the initial temperature of the cryoprotectant, and-seeding temperature (Trad FS, et al., 1999). Survival rates have been further improved with vitrification, a technique that uses high concentrations of cryoprotectant and rapid freezing by immersion in liquid nitrogen, preserving oocytes in a solid glass-like state without ice formation (Loutradi KE, et al., 2008; Oktay K, et al., 1997). With the use of intra-cytoplasmic sperm injection (ICSI), the hardened zona is not a barrier to fertilization (Polak de Fried E, et al., 1998). Survival, fertilization, and pregnancy rates achieved with cryopreserved oocytes are rap-idly improving and approaching those achieved with fresh oocytes (Grifo JA, & Noyes N, 2010; Nagy ZP, et al., 2009). A randomized comparison of results achieved with slow-freeze and vitrification observed that vitrification resulted in better oocyte survival (81% vs. 67%), fertilization (77% vs. 67%), and clinical pregnancy rates per thawed oocyte (5.2% vs. 1.7%).

A study examining outcomes achieved with vitrified donor oocytes observed 87% thaw survival, 87% fertilization, and 68% blastocyst formation, with 15/20 recipients (75%) achieving pregnancy after embryo transfer (Cobo A, Kuwayama M, Perez S, et al). Another using both slow-frozen and vitrified oocytes observed 92% survival, 79% fertilization, 42% implantation, and a 57% on going pregnancy rate (Grifo JA & Noyes N, 2010). Although the number of pregnancies and deliveries resulting from oocyte cryopreservation is still somewhat small, the number is rapidly increasing, and early perinatal outcomes data are reassuring. The incidence of chromosomal abnormalities in human embryos derived from cryopreserved oocytes is no different from that observed in control embryos derived from fresh oocytes (Gook DA, et al., 1994; Cobo A, et al., 2001). A study comparing outcomes in 200 infants derived from vitrified oocytes and in infants resulting from conventional fresh IVF found no differences in birth weight or in the incidence of birth defects (Chian RC, et al., 2008). A review of over 900 live births resulting from IVF of cryopreserved oocytes also observed no increase in the prevalence of congenital anomalies compared to that in the general population (Noyes N, et al., 2009). Oocyte cryopreservation is a viable fertility preservation strategy for women without partners seeking to preserve their fertility. Unfortunately few cancer patients have sufficient time to undergo ovarian stimulation before their treatment begins. The technology also holds enormous promise as a means to simplify oocyte donation, via egg banking, and is rapidly emerging as an elective fertility preservation strategy for women anticipating delayed childbearing and concerned about their future fertility. Currently, elective oocyte cryopreservation to defer reproductive aging is controversial, primarily because the great majority of outcomes data have come from experience with cryopreserved oocytes obtained from healthy young oocyte donors and cannot be extrapolated to older women who represent the majority of those expressing interest in elective oocyte cryopreservation (Rybak EA & Lieman HJ 2009; ASRM Practice Committee). However, when age-stratified outcomes data become available, allowing women to be accurately informed about their prognosis for success, elective oocyte cryopreservation may realistically offer women the means to set their "biological clock."

10.2.1 Ovarian tissue cryopreservation

At least in theory, ovarian tissue cryopreservation offers the means to freeze thousands of primordial follicles for later in vitro maturation or to store tissue for xenografting into an animal host or later auto transplantation (Jeruss JS, Woodruff TK., 2009). Currently, autologous transplantation of ovarian tissue seems the most practical and effective approach

because the technique has successfully restored fertility to women with ovarian failure resulting from cancer chemotherapy (Andersen CY,, et al., 2008; Demeestere I, 2006,2010; et al; Silber SJ 2009). Ovarian tissue is removed surgically via laparoscopy or laparotomy and frozen using either a slow-cool or vitrification technique, before the insult expected to result in ovarian failure. Later, it can be thawed and transplanted back into the patient in or near its original location (orthotopic transplantation) or to another site, such as the forearm or abdominal wall (heterotopic transplantation). The advantage of orthotopic transplantation is that pregnancy might be achieved without assistance, whereas heterotopic transplantation requires IVF (Jeruss JS & Woodruff TK. 2009). Live births have been achieved after transplantation of frozen-thawed ovarian tissue in sheep, (Candy CJ et al., 2000) and the first live birth in a primate after a fresh heterotopic ovarian transplantation has been reported (Lee DM, et al). Human oocytes have been obtained from heterotopic transplants and fertilized in vitro to yield embryos for transfer, resulting in a biochemical pregnancy (Rosendahl M, et al., 2006). The only human pregnancy achieved after heterotopic transplantation was achieved without assistance, indicating that the oocyte from which it arose came from the patient's existing ovary rather than from the transplant**.** Orthotopic transplantation has been successfully achieved in humans.

A number of live births have been reported after autologous orthotopic transplantation of cryopreserved ovarian tissue. Frozen ovarian tissue also has been transplanted successfully between monozygotic twin sisters after the receiving twin developed premature ovarian failure (Silber SJ, & Gosden RG, 2007). A 2008 systematic review identified 25 reports describing a total of 46 cases of ovarian tissue transplantation for treatment of premature ovarian failure or infertility, although most involved transplantation of fresh rather than frozen ovarian tissue (Bedaiwy MA, et al., 2008). The mean time to return of ovarian function was 120 days (range 60–244 days) and data were insufficient to evaluate function beyond 1 year. Fresh grafts were more likely to succeed, and in 25 women who sought pregnancy, eight conceived nine pregnancies. At least one potential risk of ovarian tissue cryopreservation and auto-transplantation is reseeding of tumor cells in women with malignancies. Future research focusing on defining patient suitability, tissue collection methods, and cryopreservation protocols is certainly warranted, but until effective techniques and the possibility for success can be defined, ovarian tissue cryopreservation will remain investigational and cannot be justified solely for the purpose of future use in healthy women.

11. References

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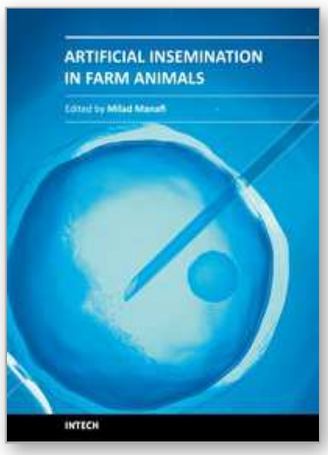

Artificial Insemination in Farm Animals

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Artificial insemination is used instead of natural mating for reproduction purposes and its chief priority is that the desirable characteristics of a bull or other male livestock animal can be passed on more quickly and to more progeny than if that animal is mated with females in a natural fashion. This book contains under one cover 16 chapters of concise, up-to-date information on artificial insemination in buffalos, ewes, pigs, swine, sheep, goats, pigs and dogs. Cryopreservation effect on sperm quality and fertility, new method and diagnostic test in semen analysis, management factors affecting fertility after cervical insemination, factors of non-infectious nature affecting the fertility, fatty acids effects on reproductive performance of ruminants, particularities of bovine artificial insemination, sperm preparation techniques and reproductive endocrinology diseases are described. This book will explain the advantages and disadvantages of using AI, the various methodologies used in different species, and how AI can be used to improve reproductive efficiency in farm animals.

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